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# Gene flow from imidazolinone-resistant domesticated sunflower to wild relatives

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Gene flow from imidazolinone (IMI)-resistant domestic sunflower to IMI-susceptible common sunflower and prairie sunflower was studied. Under greenhouse conditions, pollen from IMI-resistant domesticated sunflower was applied to flower heads of IMI-susceptible common and prairie sunflower. In addition, field studies were conducted in 2000 and 2001 near Manhattan, KS, to evaluate IMI-resistant gene flow from IMI-resistant domesticated sunflower to common and prairie sunflower under natural conditions. Common and prairie sunflower were planted in concentric circles at distances of 2.5, 5, 15, and 30 m around a densely planted IMI-resistant domesticated sunflower species. For both greenhouse and field studies, IMI-resistant gene flow was determined by treating the progeny of both wild species with 40 g ai $ha^{-1}$ of imazamox. Greenhouse crosses made by hand showed that 94% of common sunflower and 79% of prairie sunflower were resistant or moderately resistant. The resistant plants were allowed to grow in the greenhouse and were backcrossed with the corresponding susceptible wild parents. Progeny of the backcross showed a 1:1 ratio of resistant to susceptible plants. In the field, gene flow was detected up to 30 m from the pollen source for both species, and it decreased as distance from the pollen source increased. In 2000, 11 to 22% of the progeny were resistant at 2.5 m from the pollen source and 0.3 to 5% were resistant at 30 m. In 2001, the number of resistant progeny did not exceed 7 and 2% at 2.5 and 30 m from the pollen source, respectively. The results of this study showed that IMI-resistant domesticated sunflower outcrosses with common and prairie sunflower over distances typically encountered near production fields. Also, backcrosses of resistant hybrids with wild parents are successful, further increasing the potential for the spread of IMI-resistant feral sunflowers.

**Nomenclature:** Common sunflower, *Helianthus annuus*; prairie sunflower, *Helianthus petiolaris.*

**Key words:** Pollen movement, imazamox, hybridization, hybridization rates, FURD.

Herbicide-resistant crops (HRCs) are becoming increasingly important in agricultural production. They provide cost-effective and flexible weed management strategies and favor the use of herbicides with environmentally sound properties (Duke 1996). In addition, HRCs promote the use of reduced and no-till practices resulting in less soil erosion (Duke 1996; Dyer et al. 1993). In 2001, more than 40 million ha worldwide were planted with HRCs. This area represents an increase of 24% compared with 2000. Soybean [*Glycine max* (L.) Mer.], canola (*Brassica napus* L.), cotton (*Gossypium hirsutum* L.), and corn (*Zea mays* L.) account for more than 99% of the HRC area (James 2001). Despite its rapid adoption, the development of HRCs has been accompanied by several concerns, including a decrease in the number of herbicides available, increase in herbicide use, reduction in nonchemical weed control methods, weed population shifts, HRCs as volunteers in subsequent crops, and herbicide-resistance gene flow to wild species. Gene flow from HRCs to wild relatives can add herbicide resistance to these species, resulting in weeds that are more difficult to control (Ellstrand 1988; Manasse 1992; Mikkelsen et al. 1996).

Gene flow between crops and wild relatives has occurred for many years and contributed to the evolution and extinction of weed species (Barret 1983; Ellstrand et al. 1999). For example, interspecific hybridization between commercial sorghum (*Sorghum bicolor*) and wild sorghum (*Sorghum propinquum*) resulted in johnsongrass (*Sorghum halepense*), one of the worst weeds in the world. Hybridization of cultivated radish (*Raphanus sativus*) and a weedy relative resulted in wild radish (*Raphanus raphanistrum*), a major weed problem in the western United States (Panetsos and Baker 1967; Paterson et al. 1995). Many crops, including rice (*Oryza sativa*), sunflower, sugarbeet (*Beta vulgaris*), canola, barley (*Hordeum vulgare*), and wheat (*Triticum aestivum*), hybridize freely with their wild relatives (Arriola and Ellstrand 1996; Brown and Brown 1996; Brown et al. 1995; Klinger et al. 1991, 1992; Langevin et al. 1990; Ritala et al. 2002; Seefeldt et al. 1998; Snow and Moran-Palma 1996).

Herbicide-resistance gene flow from HRCs to wild relatives was reported for several crop–weed systems. Brown and Brown (1996) and Brown et al. (1995) reported that glufosinate-resistant canola can outcross with field mustard (*Brassica rapa*), producing glufosinate-resistant hybrids. Hall et al. (2000) found volunteer canola progeny resistant to glyphosate at 500 m from a glyphosate-resistant canola field. In addition, volunteer canola plants growing in fields close to glufosinate-, imidazolinone (IMI)-, and glyphosate-resistant canola showed multiple resistance to glyphosate, glufosinate, and imazethapyr. Seefeldt et al. (1998) showed that

jointed goatgrass (*Aegilops cylindrica*) crosses with imazamox-resistant wheat, producing imazamox-resistant hybrids.

A major concern associated with the HRCs is the risk of introducing fitness-related genes into wild relatives, resulting in more invasive and noxious weeds (Arriola and Ellstrand 1997; Colwell et al. 1985; Ellstrand et al. 1999; Snow and Moran-Palma 1996). However, because several studies have reported triazine-resistant biotypes to be less fit than the susceptible biotypes, some yield penalty associate with the herbicide resistance trait would be expected to occur in all herbicide-resistant plants (Mallory-Smith and Eberlein 1996). A triazine-resistant canola biotype produced less biomass and up to 20% less seed yield than susceptible biotypes (Forcella 1987). Seed germination and yield of triazine-resistant foxtail millet (*Setaria italica*) were reduced by 22 and 50%, respectively, compared with susceptible plants (Darmency and Pernes 1989). Furthermore, triazine-resistant canola yielded 20 to 30% less than conventional varieties (Beversdorf et al. 1988). Also, triazine-resistant biotypes of redroot pigweed (*Amaranthus retroflexus*) and common groundsel (*Senecio vulgaris*) were less competitive than susceptible biotypes (Conard and Radosevich 1979; Holt 1988). In contrast, Holt and Thill (1994) reported that growth and productivity were not different between populations of dinitroaniline-resistant and -susceptible goose grass (*Eleusine indica*). Alcocer-Ruthling et al. (1992) and Dyer et al. (1993) reported that canopy height, plant biomass, and seed yield of sulfonylurea-susceptible and -resistant prickly lettuce (*Lactuca serriola*) and kochia (*Kochia scoparia*) biotypes were similar. Marshall et al. (2001) found no difference in photosynthesis, leaf area, height, and dry weight between imazethapyr-resistant and -susceptible common sunflower.

Resistance to IMI herbicides has been recently introduced into domesticated sunflower through conventional breeding methods (Al-Khatib and Miller 2000; Al-Khatib et al. 1998; Miller and Al-Khatib 2002). This IMI-resistant gene was derived from naturally occurring IMI-resistant common sunflower (Al-Khatib et al. 1998). Imazamox-resistant commercial hybrids are currently under development and will be released to sunflower growers in 2003 (BASF 2001).

Domestic sunflower is native to North America, with about 50 wild species growing near fields planted with domesticated sunflower (Schilling and Heiser 1981). Therefore, the commercial release of an imazamox-resistant domesticated sunflower variety will likely result in transfer of the herbicide-resistance trait to wild relatives. This risk of gene flow is further exacerbated because inadvertent spread of pollen carrying resistance genes is much greater in sunflower as an insect pollinated outcrossing species than as a self-pollinated species. In addition, wild sunflowers possess genetic and floral characteristics that facilitate successful hybridization with domestic sunflower (Arias and Rieseberg 1994). The likelihood of spontaneous hybridization between domesticated sunflower and wild relatives is also favored by overlapping flowering periods of domesticated and most of the wild sunflower species, shared pollinators, self-incompatibility of the wild species, and diploidy (Keeler and Turner 1990; Rogers et al. 1982; Schilling and Heiser 1981). Moreover, crop × wild sunflower hybrids are very likely to backcross with the wild species and to transfer the resistance gene to a wild relative more easily than the resistant domesticated species (Snow et al. 1998).

Physical distance, chromosomal structural differences between species, and interspecific pollen competition have been suggested as significant barriers to gene flow between sunflower species (Arias and Rieseberg 1994; Rieseberg et al. 1995a, 1995b, 1999). However, pollen can be transferred from crop to wild sunflower plants as far away as 1,000 m, indicating that an isolation zone is unlikely to prevent hybridization, and mixed loads of self- and heterospecific pollens do not appear to affect frequency of interspecific hybridization (Arias and Rieseberg 1994; Desrochers and Rieseberg 1998).

The objectives of this study were to (1) determine the outcrossing rates between the IMI-resistant domesticated sunflower and two wild relatives—common sunflower and prairie sunflower and (2) determine the outcrossing rates between the progeny resulting from crosses of IMI-resistant domesticated sunflower × wild species hybrids with the corresponding susceptible wild species parent.

## Materials and Methods

### Plant Material

Common sunflower and prairie sunflower were used in this study because they occur in close proximity to domesticated sunflower throughout the central and western United States. Both species are annual, self-incompatible, and diploid (n = 17) (Seiler and Rieseberg 1997).

Common sunflower achenes were collected from plants growing near the Konza Prairie Research Natural Area in northeast Kansas, where no herbicide had been applied in the past 25 yr; achenes of prairie sunflower were obtained from the USDA-ARS North Central Regional Plant Introduction Station at Ames, IA. The IMI-resistant domesticated sunflower hybrid HA 425/RHA426 was provided by the USDA-ARS Sunflower Research Unit of the Crop Science Laboratory at Fargo, ND.

Achenes from common sunflower and prairie sunflower were surface sterilized with a 10% sodium hypochlorite solution for 20 min and rinsed thoroughly with distilled water before being scarified by removing approximately 2 mm of seed coat from the widest portion of each achene. To interrupt dormancy, scarified achenes were placed on paper towels moistened with 0.3 μM gibberellic acid solution and incubated in the dark at 25 ± 1 C for 24 to 48 h. Immediately after germination, seed coats were removed, and seedlings were placed on new paper towels moistened with distilled water (Al-Khatib et al. 1998). After the expansion of cotyledons and roots, the seedlings were incubated in a growth chamber for 48 h. The growth chamber conditions were 25 and 20 C day and night temperature and 16 and 8 h day and night photoperiod, respectively and photosynthetic photon flux (PPF) of 550 ± 20 μmol $m^{-2}$ $s^{-1}$.

Seedlings were then transplanted into 33-cm-diam pots filled with a 1:1 (v/v) mixture of soil and sand. The soil was a Morrill loam (mesic typic Argiudolls) with pH 7.0 and 1.7% organic matter. Plants were fertilized weekly with a solution containing 300 μg $L^{-1}$ N, 250 μg $L^{-1}$ P, and 220 μg $L^{-1}$ K. Plants were grown in greenhouse conditions. The growing conditions were 25 and 20 C day and night temperature and 16 and 8 h day and night photoperiod, re-

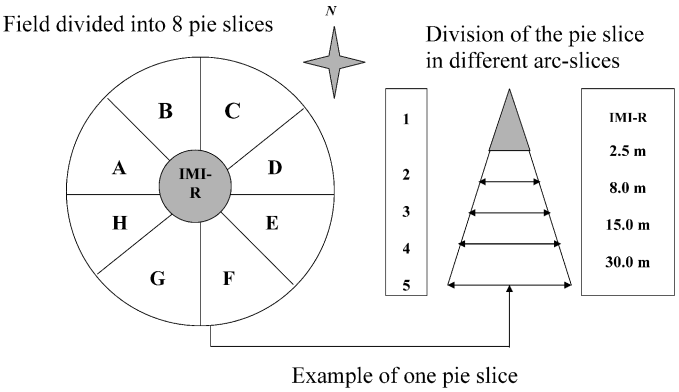


FIGURE 1. Harvest diagram of each field location. Each location was divided into eight slices (A through H) and each slice divided into four arc slices corresponding to distances from the IMI-resistant pollen source.

spectively. Supplemental light was provided at 80 μmol $m^{-2}$ $s^{-1}$ PPF. IMI-resistant domesticated sunflower was planted directly into 33-cm-diam pots. Soil and greenhouse conditions were as described earlier.

## Greenhouse Study

To determine primary gene flow from domesticated sunflower to wild species, 10 flower heads per common sunflower and prairie sunflower plant were randomly selected and crossed by hand with pollen from IMI-resistant domesticated sunflower (Fick 1978). Pollen was collected from heads of domesticated sunflower into paper bags and applied gently with a brush to the surface of the stigmata of the wild species. Pollen was applied twice to the same flower head to ensure pollination. The second pollination occurred 48 h after the first. Pollinated heads were marked and then harvested at physiological maturity. Heads were threshed and achenes counted.

A sample of 1,500 achenes resulting from cross of common and prairie sunflower with IMI-resistant domesticated sunflower was germinated and grown in 15-cm-diam pots as described above. At the two- to three-leaf stage, plants (hereafter referred as $F_1$ hybrids) were treated with 40 g $ha^{-1}$ imazamox plus 0.25% (by volume) nonionic surfactant.[1] Herbicide was applied with a bench-type[2] sprayer equipped with an 80015LP tip[3] and calibrated to deliver 187 L $ha^{-1}$ at 138 kPa.

Imazamox visual injury was estimated 14 d after treatment (DAT) on a scale of 0 to 100%, where 0 indicates no injury, and 100% indicates mortality. Transfer of imazamox resistance to susceptible populations was used to detect outcrossing. The outcrossing rate was calculated for each species as the proportion of $F_1$ individuals with less than 80% injury compared with the total treated progeny. The $F_1$ plants of common and prairie sunflower with less than 20% injury were transplanted into 33-cm-diam pots and grown as described earlier. Pollen was collected from these plants and used to pollinate their corresponding IMI-susceptible wild parents to determine the secondary gene flow from $F_1$ hybrids to wild parents. Resistance to imazamox for progeny of the backcross (hereafter referred as $BC_1$) and outcross frequency were evaluated as described above. The experimental design was a randomized complete block with four replications, and experiments were conducted twice. A chi-square test was performed for both common sunflower and prairie sunflower to estimate the resistance to susceptible segregation ratios of $BC_1$ plants.

An imazamox dose–response study was conducted to evaluate the resistance level of $F_1$ hybrids and $BC_1$ plants to imazamox. $F_1$ hybrids and $BC_1$ seedlings were planted in 15-cm-diam pots. At the four-leaf stage, seedlings were treated with 0, 10, 20, 40, 80, and 160 g of imazamox $ha^{-1}$, corresponding to 0, 0.25, 0.5, 1, 2, and 4 times the recommended use rate of imazamox, respectively. At 14 DAT, visible injury was rated as described earlier. Pots were arranged in a randomized complete block design with four replications, and experiments were conducted twice. Visible injury ratings of both $F_1$ and $BC_1$ plants were subjected to nonlinear logistic analysis (Seefeldt et al. 1995). The herbicide rate required to cause 50% injury ($GR_{50}$) was determined, and the R:S (resistant–susceptible) ratio was calculated.

## Field Study

Field experiments were conducted in 2000 and 2001 at the Kansas State University Agronomy Department Research Farm at Manhattan, KS, and at the Ashland Bottoms Research Farm located 12 km south of Manhattan, KS. At Manhattan, KS, the soil type was a Smolan silt loam (fine, montmorillonitic mesic Patchic Argiustoll) with pH of 6.1 and 6.5 and 2.6 and 2.9% organic matter in 2000 and 2001, respectively. At Ashland Bottoms, the soil was a Hay-

TABLE 1. Percentage of hand-pollinated heads producing filled achenes, achenes per head, and percent achene germination for common and prairie sunflower in primary and secondary gene flow studies.[a]

| Crosses (♂ × ♀) | Heads with achenes | No. of achenes per head | Germination[b] |
|---|---|---|---|
| | % | | % |
| Primary gene flow | | | |
| IMI-[c]resistant domesticated sunflower × common sunflower | 21 b | 62 a | 47 a |
| IMI-resistant domesticated sunflower × prairie sunflower | 11 c | 18 b | 28 b |
| Secondary gene flow | | | |
| Common sunflower $F_1$ hybrid × common sunflower | 31 a | 17 b | 21 b |
| Prairie sunflower $F_1$ hybrid × prairie sunflower | 17 c | 19 b | 6 c |

[a] Values followed by the same letter within a column are not significantly different at P = 0.05.
[b] Percentage of germination from a sample of 1,500 achenes.
[c] Abbreviation: IMI, imidazolinone.

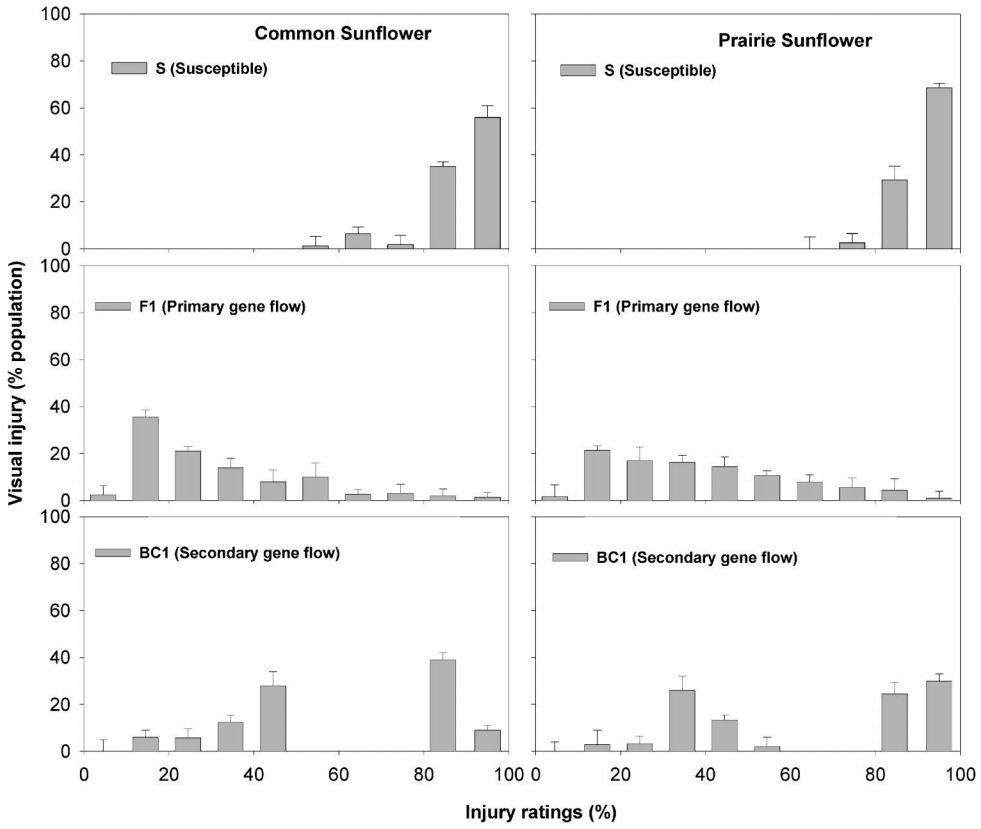


FIGURE 2. Frequency distribution of imazamox injury of common sunflower and prairie sunflower plants from the primary gene flow ($F_1$) and secondary gene flow ($BC_1$) compared with the frequency distribution of injury in susceptible plants (S).

nie sandy loam with a pH of 6.1 and 3.3% organic matter for both years. The field at the Manhattan site was previously planted with oat (*Avena fatua* L.) in 2000 and wheat in 2001. The Ashland Bottoms field was previously planted with sorghum.

Achenes of common and prairie sunflower were germinated and grown in the greenhouse as described earlier. At the four- to six-leaf stage, common and prairie sunflower plants were transplanted into the field in concentric circles at distances of 2.5, 5.0, 15.0, and 30.0 m from the outside border of a central 20-m-diam circle. Plants were assigned in sets of one common sunflower and one prairie sunflower plant separated by 1.5 m. Within the circle, plant sets were separated from each other by a 3-m-wide gap.

Domesticated IMI-resistant sunflower was planted in the central 20-m-diam circle 3 and 4 wk after the wild species were planted in 2000 and 2001, respectively, to ensure the overlap of flowering. Sunflower rows were 76 m wide, and seeds were placed 15 cm apart. Plants were watered as needed for the first 3 wk. Flowers of common and prairie sunflower that appeared before and after the flowering stage of the IMI-resistant domesticated sunflower were removed.

Fields were divided into eight equal slices corresponding to each of the compass direction points. Each slice included four arc slices corresponding to each of the distances evaluated, and each distance contained eight (intracircle) arc slices (Figure 1). At maturity, heads of common and prairie sunflower were harvested separately from each arc slice and threshed, and the achenes were collected.

A 50-g subsample of common and prairie sunflower achenes from each arc-slice sample of each location was germinated as described earlier. Seedlings were transplanted into 13- by 23-cm pots filled with 2.5 kg of soil. Soil type and greenhouse growth conditions were as described earlier. Plants were thinned to 10 per pot. The experiment was a randomized complete block with four replications. The experiment was conducted twice.

At the two- to three-leaf stage, common and prairie sunflower plants were treated with 40 g $ha^{-1}$ of imazamox as described earlier. Outcrossing frequency was calculated as the proportion of plants rated 80% or less for imazamox injury. Percentage of imazamox-resistant progeny for each arc-slice sample was compared with the natural mutation rate of $1 \times 10^{-6}$ for a single dominant nuclear gene (Maxwell and Mortimer 1994) according to a modified one-tailed test for binomial percentage (Ott 1993):

$$RF \geq \left[\mu + 1.645\sqrt{\frac{\mu(1-\mu)}{n}}\right] \times 100 \qquad [1]$$

where RF is the percentage of imazamox-resistant progeny found in each arc-slice sample; $\mu$ is the natural mutation rate of $1 \times 10^{-6}$ for a single, dominant nuclear gene; and $n$ is the total number of plants in each pot. The first unnatural resistance distance (FURD) measures the furthest distance at which the calculated RF from each arc-slice sample was significantly greater than the background mutation rate (Marshall et al. 2001). A single FURD was obtained from each slice. The FURD values were used as response variables in analysis of variance to compare slice orientations, and means were compared at the $P = 0.05$ level. At each location, the percentage of imazamox resistance at each

TABLE 2. Segregation ratios between imazamox-resistant (R) and -susceptible plants (S) of common sunflower and prairie sunflower from the secondary gene flow ($BC_1$).

| Species | Segregation | R:S ratio | Chi-squared | P |
|---|---|---|---|---|
| Common sunflower | 164 R:151 S | 1:1 | 0.54 | 0.46 |
| Prairie sunflower | 41 R:49 S | 1:1 | 0.71 | 0.52 |

arc slice was averaged across north and south sections to illustrate the effects of distance, location, and wind direction. Means of percentage of imazamox resistance at each arc slice were compared using LSD at $P = 0.05$.

## Results and Discussion

### Greenhouse Study

In the primary gene flow study, from the total of 800 pollinated heads of each species, 21% of common sunflower and 11% of prairie sunflower produced filled achenes; in the secondary gene flow study, these values were 31% for common sunflower and 17% for prairie sunflower (Table 1). Most of the filled achenes were on the periphery of the flower head, whereas the empty achenes generally were in the center of the flower heads. This is in agreement with earlier studies that showed when the head diameter increases, achenes in the center of the head failed to develop (Seiler 1997).

In the primary gene flow study, common sunflower produced more achenes per head than prairie sunflower. However, achenes per head between these two species did not differ in the secondary gene flow. Common sunflower produced four times more achenes per head during primary gene flow compared with secondary gene flow, whereas the number of achenes per head in prairie sunflower did not differ between primary and secondary gene flow (Table 1). In addition, common sunflower produced larger achenes in the secondary gene flow than in the primary gene flow (data not shown). This is possible because sunflowers have an ability to compensate for reduction in number of achenes produced per head by increasing the weight of individual achenes (Seiler 1997). Furthermore, $F_1$ plants were treated with imazamox to screen for resistance, and this affected their growth and development as well as pollen production. Therefore, viability of pollen from the $F_1$ plants may have affected pollination in the secondary gene flow study (Chandler et al. 1986; Seiler 1997).

In general, seed germination was higher in common sunflower than in prairie sunflower and in achenes resulting from the primary gene flow than in achenes from the secondary flow (Table 1). This suggests that crop genes incorporated in $F_1$ plants contributed to lower dormancy and consequently higher germination of achenes, but that effect was lost with the backcross, resulting in lower germination of $BC_1$ achenes (Snow et al. 1998).

The $F_1$, $BC_1$, and IMI-susceptible plants of common and prairie sunflower differed in their response to imazamox (Figure 2). Although $F_1$ plants showed a wide range of symptoms in response to imazamox, the response of feral populations of common and prairie sunflower was concentrated at the susceptible end of the rating scale, with overall injury ratings greater than 80%. Therefore, in this study, all $F_1$ and $BC_1$ plants rated 80% or lower for imazamox injury were considered resistant. However, because a wide range of injury was observed, plants were categorized into three levels of resistance: (1) resistant if injury was lower than 20%, (2) moderately resistant if the injury rating was between 20 and 80%, and (3) susceptible if the injury rating was over 80%.

Of the 705 common sunflower and 420 prairie sunflower progeny from the primary gene flow study, 38 and 23% showed less than 20% injury, respectively (Figure 2). In these plants, injury symptoms 14 DAT were slight chlorosis, but symptoms faded 21 DAT. In addition, 56% of common sunflower and 66% of prairie sunflower showed 20 to 80% injury. At 14 DAT, symptoms on these plants included slight to severe chlorosis of the growing point and plant stunting. At 21 DAT, these symptoms faded, and regrowth developed from lateral buds. At 14 DAT, 6% of common sunflower and 11% of prairie sunflower had more than 80% injury. Symptoms included severe chlorosis and necrosis on the growing point and severe plant stunting. These plants were unable to recover from imazamox injury.

In the secondary gene flow study, 12 and 4% of common and prairie sunflower $BC_1$ progeny were resistant, respectively. However, 40% of common and 41% of prairie sunflower were moderately resistant. In addition, 48% of common sunflower and 55% of prairie sunflower plants were killed or severely injured by imazamox (Figure 2). A chi-square test showed a highly significant 1:1 segregation between the susceptible and nonsusceptible $BC_1$ plants (Table 2).

The outcrossing rate as indicated by plants resistant to imazamox was 92% for common and 90% for prairie sunflower in the primary gene flow study and 52 and 46% for

TABLE 3. Outcrossing rates for common and prairie sunflower in primary and secondary gene flow studies.

| Crosses (♂ × ♀) | Outcrossing rate[a] |
|---|---|
| | % |
| Primary gene flow | |
| IMI-[b]resistant domesticated sunflower × common sunflower | 92 ± 5 |
| IMI-resistant domesticated sunflower × prairie sunflower | 90 ± 8 |
| Secondary gene flow | |
| Common sunflower $F_1$ hybrid × common sunflower | 52 ± 3 |
| Prairie sunflower $F_1$ hybrid × prairie sunflower | 46 ± 9 |

[a] Outcrossing rate was calculated as the proportion of plants with less than 80% injury at 14 d after treatment with 40 g $ha^{-1}$ imazamox compared with the total plants treated.

[b] Abbreviation: IMI, imidiazolinone.

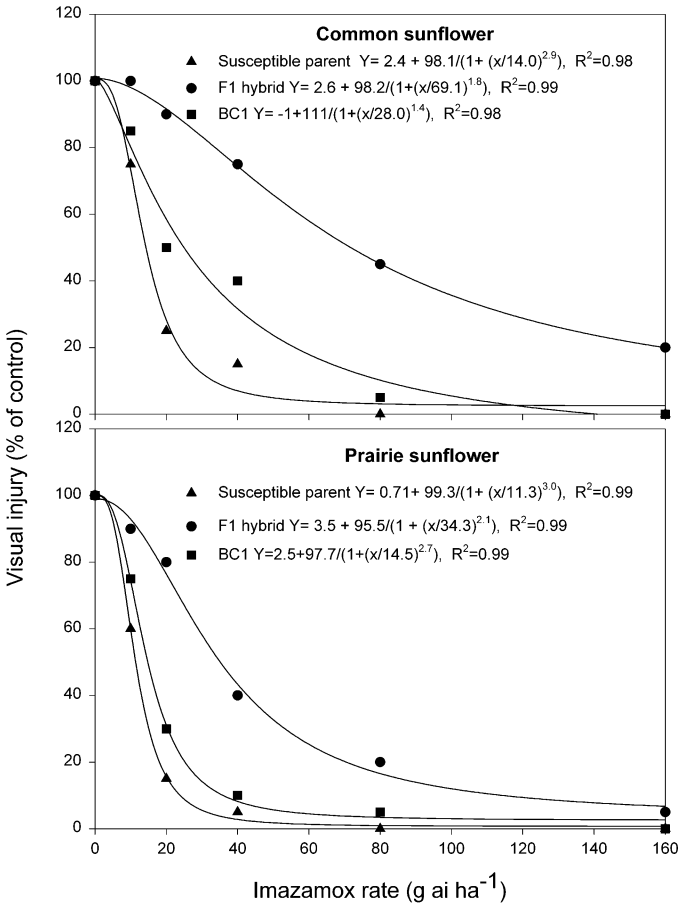


FIGURE 3. Imazamox dose–response curves of $F_1$, $BC_1$, and IMI-susceptible common sunflower and prairie sunflower plants.

common and prairie sunflower, respectively, in the secondary gene flow study (Table 3).

Hall et al. (2000) reported that resistance to imazamox is conferred by two semidominant genes, and the presence of either gene was sufficient to confer resistance. In contrast, Bruniard and Miller (2001) suggested that IMI resistance is controlled by a major gene (Imir1) with a semidominant effect and a second gene (Imir2) with a modifying effect when the major gene is present. Full resistance is only achieved by homozygosity of both genes (Imir1 Imir1, Imir2 Imir2). Therefore, heterozygous for both genes (Imir1 imir1, Imir2 imir2), should be partially resistant. However, 6% of common sunflower plants and 11% of prairie sunflower plants were susceptible. A likely explanation is that these plants resulted from self-pollination of their corresponding parents instead of from cross-pollination with the IMI-resistant domesticated sunflower. Although common and prairie sunflower are self-incompatible, selfing can be induced in the presence of a mixture of self- and heterospecific pollen (Desrochers and Rieseberg 1998; Rieseberg et al. 1998). Furthermore, the difference between plants, as expressed by differences in injury ratings and symptoms, suggests that both resistance genes were expressed in the resistant plants, whereas in the moderately resistant plants, the expression of the modifier gene may have been affected by the genetic background of the wild species.

The dose–response curves show that injury rating of susceptible common and prairie sunflower was higher than 80% at the recommended rate of 40 g ai $ha^{-1}$ of imazamox. Injury ratings for $F_1$ plants receiving the recommended dose were 25 and 60% for common and prairie sunflower plants, respectively (Figure 3). The $GR_{50}$ were 71.8 and 14.3 g ai $ha^{-1}$ for common sunflower $F_1$ progeny and the susceptible parent, respectively, and 35.1 and 11.4 g ai $ha^{-1}$ for the prairie sunflower $F_1$ progeny and the susceptible parent, respectively. The R:S ratios indicate that the common and prairie sunflower $F_1$ plants were five and three times more resistant than their corresponding susceptible parents, respectively. In $BC_1$ plants, resistance to imazamox in common sunflower was 2.2 times greater than in susceptible plants. Prairie sunflower plants were 1.3 times more resistant than the susceptible plants. This indicates that $F_1$ IMI-resistant hybrids of common and prairie sunflower backcrossed with the corresponding susceptible parent, producing resistant plants. The backcross would provide a bridge

TABLE 4. First unnatural resistance distance (FURD) as influenced by direction and distance of common sunflower and prairie sunflower from imidazolinone-resistant domesticated sunflower at Manhattan and Ashland Bottoms, KS, in 2000 and 2001.

| | FURD | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Common sunflower | | | | Prairie sunflower | | | |
| | 2000 | | 2001 | | 2000 | | 2001 | |
| Slice[a] | Manhattan | Ashland Bottoms | Manhattan | Ashland Bottoms | Manhattan | Ashland Bottoms | Manhattan | Ashland Bottoms |
| | m | | | | | | | |
| North circle section | | | | | | | | |
| A | 7.9 ± 1.7 | 8.3 ± 2.3 | 5.3 ± 2.1 | 8.2 ± 1.7 | 8.9 ± 2.1 | 9.3 ± 2.1 | 4.3 ± 1.3 | 7.2 ± 1.6 |
| B | 6.0 ± 1.7 | 7.7 ± 2.3 | 4.6 ± 2.1 | 6.7 ± 1.7 | 8.9 ± 2.1 | 9.7 ± 2.1 | 5.8 ± 1.3 | 9.3 ± 1.6 |
| C | 7.5 ± 1.7 | 8.4 ± 2.3 | 4.3 ± 2.1 | 7.6 ± 1.7 | 8.2 ± 2.1 | 12.2 ± 2.1 | 7.3 ± 1.3 | 10.1 ± 1.6 |
| D | 8.2 ± 1.7 | 9.1 ± 2.3 | 6.2 ± 2.1 | 3.5 ± 1.7 | 8.0 ± 2.1 | 9.8 ± 2.1 | 8.2 ± 1.3 | 9.7 ± 1.6 |
| South circle section | | | | | | | | |
| E | 4.6 ± 1.7 | 6.6 ± 2.3 | 5.7 ± 2.1 | 6.1 ± 1.7 | 7.5 ± 2.1 | 6.8 ± 2.1 | 4.7 ± 1.3 | 6.6 ± 1.6 |
| F | 5.9 ± 1.7 | 8.6 ± 2.3 | 4.1 ± 2.1 | 7.8 ± 1.7 | 9.5 ± 2.1 | 6.0 ± 2.1 | 5.8 ± 1.3 | 5.5 ± 1.6 |
| G | 5.9 ± 1.7 | 6.4 ± 2.3 | 5.4 ± 2.1 | 6.8 ± 1.7 | 7.0 ± 2.1 | 5.5 ± 2.1 | 6.3 ± 1.3 | 6.3 ± 1.6 |
| H | 8.0 ± 1.7 | 6.9 ± 2.3 | 6.1 ± 2.1 | 5.9 ± 1.7 | 5.8 ± 2.1 | 6.1 ± 2.1 | 5.6 ± 1.3 | 5.4 ± 1.6 |

[a] Study layout in Figure 1.

TABLE 5. Percent imazamox resistance in progeny of common and prairie sunflower planted at different distances from imidazolinone-resistant domesticated sunflower at Manhattan and Ashland Bottoms, KS, in 2000 and 2001.

| | Common sunflower | | | | Prairie sunflower | | | |
|---|---|---|---|---|---|---|---|---|
| | 2001 | | 2001 | | 2000 | | 2001 | |
| Distance from the pollen source | Manhattan | Ashland Bottoms | Manhattan | Ashland Bottoms | Manhattan | Ashland Bottoms | Manhattan | Ashland Bottoms |
| m | % | | | | | | | |
| North circle section | | | | | | | | |
| 2.5 | 10.9 | 14.1 | 6.9 | 5.6 | 16.5 | 22.2 | 2.5 | 3.8 |
| 5.0 | 10.5 | 7.2 | 3.8 | 1.8 | 8.7 | 12.5 | 3.1 | 1.9 |
| 15.0 | 3.8 | 3.8 | 1.2 | 1.2 | 4.5 | 9.7 | 2.5 | 0.6 |
| 30.0 | 0.6 | 1.9 | 1.8 | 1.2 | 1.5 | 5.6 | 0.6 | 0.0 |
| LSD (0.05) | 1.8 | 1.6 | 1.5 | 1.4 | 1.8 | 2.0 | 1.6 | 1.3 |
| South circle section | | | | | | | | |
| 2.5 | 13.1 | 8.7 | 1.9 | 1.3 | 13.9 | 13.1 | 2.5 | 1.2 |
| 5 0 | 7.5 | 4.2 | 0.0 | 0.6 | 6.9 | 7.5 | 1.8 | 0.6 |
| 15.0 | 8.1 | 2.3 | 1.2 | 0.0 | 3.4 | 8.1 | 0.6 | 0.0 |
| 30.0 | 0.3 | 0.3 | 0.6 | 0.6 | 0.3 | 0.3 | 0.0 | 0.0 |
| LSD (0.05) | 1.8 | 1.6 | 1.5 | 1.4 | 1.8 | 2.0 | 1.6 | 1.3 |

for low-frequency spread of the gene in wild populations (Snow et al. 1998; Whitton et al. 1997).

## Field Study

The FURD ranged from 4.6 to 9.1 m and from 3.5 to 7.8 m in common sunflower in 2000 and 2001, respectively. In prairie sunflower, FURD ranged from 5.5 to 12.2 m and from 4.3 to 10.1 m in 2000 and 2001, respectively (Table 4). These results indicate that resistance to imazamox was transferred from IMI-resistant domesticated sunflower to common and prairie sunflower at a level significantly greater than the expected background mutation rate.

FURD values differed between the north and south sections of the fields, therefore data are reported separately by sections of the circles (Table 4). Overall, greater FURD values were observed in the north than in the south sections of the circles, suggesting that movement of pollen was affected by the predominant wind direction from the south. This effect was more prominent in 2000 than in 2001 and in Ashland Bottoms than in Manhattan. Sunflower are insect pollinated species. Nevertheless, pollination is affected by wind direction (Marshall et al. 2001; Miller 1987).

The number of IMI-resistant individuals of both sunflower species decreased as the distance from the source of the resistant pollen increased (Table 5). For example, in 2000, IMI resistance in common sunflower ranged from 14.1% at 2.5 m to 0.3% at 30 m from the pollen source. In prairie sunflower, resistance ranged from 22% at 2.5 m to 0.3% at 30 m from the pollen source. In 2001, the resistance ranged from 6.9% at 2.5 m to 0.6% at 30 m for common sunflower, whereas it ranged from 3.8% at 2.5 m to 0% at 30 m from prairie sunflower. Although resistance to imazamox was detected in both common sunflower and prairie sunflower at all locations, higher levels of resistance occurred in 2000 than in 2001. Strong winds in 2000 favored pollination; whereas in 2001, rain during the flowering period hindered pollination, contributing to lower levels of resistance. In general, imazamox resistance was higher at Ashland Bottoms than at Manhattan. The Ashland Bottoms studies were surrounded by soybean, corn, and sorghum fields. These provided a more attractive environment for occurrence of insects than the wheat and oat fields surrounding the Manhattan studies. Over all locations and years, larger percentage of plants with resistance were observed in prairie than in common sunflower (Table 5). Prairie sunflower plants were shorter than common sunflower, and this may have facilitated movement of pollinators between the crop and the species. In addition, pollinators may have been attracted to prairie sunflower in response to its stronger aroma and larger number of flowers.

This study showed that IMI-resistant domesticated sunflower can outcross with common and prairie sunflower, producing IMI-resistant plants. However, the outcrossing rates were higher in the greenhouse than in the field, suggesting that outcrossing in the field was affected by occurrence of pollination among the wild species and by presence of other floral choices for pollinators. Sunflower pollen can travel as far as 1,000 m (Arias and Rieseberg 1994), which indicates strong potential for spread of resistance in this species. This study indicates a high potential for the spread of resistance and the production of fertile hybrids that will become a source of successful secondary gene flow. Although backcrosses with the wild parents can occur, the proportion of IMI-resistant individuals in wild populations may not increase in the absence of herbicide pressure. Nevertheless, successful secondary gene flow indicates a potential for long-term establishment of the resistant trait in wild populations. The introgression, spread, and persistence of the IMI-resistant trait in wild populations will depend on the fitness of the resistant feral plants (Whitton et al. 1997).

The introduction of an IMI-resistant domesticated sunflower will provide producers with a new weed control tool. However, to ensure the long-term viability of this technology, the release of the IMI-resistant domesticated sunflower has to be accompanied by sound stewardship programs and incorporated into integrated weed management strategies that include crop rotation and rotation of herbicides of different mode of action.

## Sources of Materials

[1] X-77, a mixture of alkylarylpolyoxyetylene glycols, free fatty acids, and isopropanol. Loveland Industries Inc., P.O. Box 1289, Greeley, CO 80632.

[2] Research Track Sprayer, DeVries Manufacturing, RR1 Box 184, Hollandale, MN 56045.

[3] 80015LP TeeJet Tip, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

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